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Smokeless tobacco as a risk factor for oral squamous cell carcinoma

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Abstract:

Oral squamous cell carcinoma (OSCC) is a major oral malignancy in developing countries like India, where smokeless tobacco use is widespread and strongly linked to oral carcinogenesis. This hospital-based observational case-control study included 200 participants; comprising 100 histopathologically confirmed OSCC patients and 100 healthy controls and assessed the association of smokeless tobacco use with demographic variables, duration and frequency of use, lesion site and histopathological grade. Statistical analysis showed a significant association between smokeless tobacco use and OSCC occurrence ($p < 0.001$), with buccal mucosa being the most commonly affected site among users. Poorly differentiated carcinoma and advanced disease stage were significantly associated with longer duration and higher frequency of smokeless tobacco use. Thus, data shows that smokeless tobacco is a strong etiological risk factor for OSCC and highlights the need for targeted public health measures and tobacco cessation interventions.

Keywords: Oral squamous cell carcinoma (OSCC), smokeless tobacco, gutkha, khaini, oral cancer, carcinogenesis, tobacco chewing, oral mucosa

Background:

Oral squamous cell carcinoma (OSCC) is the most prevalent type of oral malignancy and is responsible for almost 90% of all oral cancers globally. Oral cancer is a major public health problem, particularly in South Asian countries like India, where the use of chewing tobacco products is common and accepted, accounting for one of the highest cancer burden among men [1, 2]. Smokeless tobacco (SLT) is a type of tobacco that is not burned and typically chewed, sucked or held in the mouth for extended periods of time. Smokeless tobacco products used in India include gutkha, khaini, zarda, mawa, pan masala with tobacco and betel quid with tobacco containing multiple carcinogenic compounds like tobacco specific nitrosamines (TSNAs), polycyclic aromatic hydrocarbons, aldehydes, heavy metals and reactive oxygen species which can induce epithelial dysplasia and malignant transformation, as shown by Khan et al who found that smokeless tobacco users had significantly higher risk of developing oral cavity carcinoma than non-users, thus highlighting the carcinogenic potential of chewing tobacco habits [3-5]. Oral potentially malignant disorders like leukoplakia, erythroplakia, oral submucous fibrosis and tobacco pouch keratosis are common with chronic use of smokeless tobacco and have high malignant transformation potential [6]. In recent years, several epidemiological studies have reported the strong association between consumption of smokeless tobacco

and OSCC. In a recent systematic review of systematic reviews, Asthana *et al.* found that smokeless tobacco users have significantly higher risk of oral cancer, regardless of geographic region or gender [3]. Recent Indian studies also showed that chewing of gutkha and betel quid with tobacco is a major independent risk factor for the occurrence of OSCC among younger population [7-9]. Sultana *et al.* also showed that there is a strong association between tobacco chewing habits and OSCC occurrence in South Asian populations [7]. There are significant differences among commercially available smokeless tobacco products in terms of their carcinogenic and addictive properties. The molecular pathways involved in tobacco-associated oral carcinogenesis include p53 mutation, EGFR overexpression, cyclin dysregulation and epigenetic alterations that can lead to the initiation and progression of oral cancer [10, 11]. The statistics from GLOBOCAN 2022 revealed alarming trends of increasing incidence and mortality rates of head and neck cancers in India, which were directly attributed to the use of smokeless tobacco and areca nut consumption by Goyal *et al.* [12]. The location of OSCC often matches with the location of the tobacco quid in the oral cavity. Although new diagnostic and therapeutic options have emerged, early diagnosis is still challenging because of delayed seeking of healthcare and social stigma and many patients are diagnosed at late stages as a result of the lack of awareness of the condition [12]. Early diagnosis is

difficult for chronic smokeless tobacco users as well, because the affected areas are not routinely examined [6]. Therefore, it is of interest to evaluate the role of smokeless tobacco in the development of oral squamous cell carcinoma and to determine its relationship with the severity of the disease, distribution of lesions and histopathological grading.

Materials and Methodology:

The study was an observational case-control study carried out in the Department of Oral Medicine and Pathology with the Department of Oncology of a tertiary care teaching hospital for 18 months with the approval of the Institutional Ethics Committee. The study involved 200 participants.

The study population consisted of two groups:

- [1] Group I: 100 histopathologically confirmed cases of oral squamous cell carcinoma.
- [2] Group II: 100 age and sex matched healthy controls without oral malignant lesions.

The study included patients diagnosed with OSCC who were older than 18 years of age and were willing to participate in the study. Patients were excluded if they had recurrent oral carcinoma, history of radiotherapy or chemotherapy, metastatic lesions, severe systemic illness and incomplete records. Detailed demographic data such as age, gender, occupation, socioeconomic status, educational level and locality of residence were collected.

A complete history of smokeless tobacco use was collected, including:

- [1] Type of smokeless tobacco used
- [2] Duration of habit
- [3] How often do you eat per day? How many times a day do you eat?
- [4] The location where the device is placed in the mouth.
- [5] Any habits that are related to the use of the product such as smoking and drinking alcohol

Clinical examination of oral cavity was performed under adequate illumination. The characteristics of the lesions were recorded such as site, size, ulceration, induration and lymph node involvement. Biopsy specimens stained with hematoxylin and eosin were used to obtain histopathological confirmation of OSCC. Tumors were classified as well differentiated, moderately differentiated or poorly differentiated squamous cell carcinoma. The data were entered into Microsoft Excel and analyzed using SPSS software version 25. The quantitative variables were presented as mean $\pm$ standard deviation, while the qualitative variables were presented as frequencies and percentages. Where appropriate, the following tests were used: chi-square test and

Student's t-test. The p-value of < 0.05 was regarded as statistically significant.

Results:

A total of 200 participants were included in the study comprising 100 OSCC patients and 100 healthy controls. The mean age among OSCC patients was 52.6 ± 11.4 years, whereas the control group showed a mean age of 47.2 ± 10.8 years. Male predominance was observed in both groups, with males constituting 76% of OSCC patients and 71% of controls. Most patients belonged to lower socioeconomic strata and rural backgrounds. Smokeless tobacco consumption was significantly higher among OSCC patients compared to controls. Among the OSCC group, 84% reported regular use of smokeless tobacco products, while only 38% of controls had a similar habit. The association between smokeless tobacco use and OSCC was statistically highly significant ($p < 0.001$). Gutkha was identified as the most frequently consumed product followed by khaini and betel quid with tobacco (**Table 1**). The findings indicate a significantly greater prevalence of smokeless tobacco habits among OSCC patients. Gutkha consumption demonstrated the strongest association with oral carcinoma. Combined smoking and chewing habits further increased cancer prevalence. Duration of smokeless tobacco use was strongly associated with OSCC occurrence. Among OSCC patients, 57.1% had a chewing habit exceeding 10 years, while only 18.4% of controls reported similar duration. Frequency of consumption was also significantly higher among cancer patients. Buccal mucosa emerged as the most common anatomical site affected, followed by gingivobuccal sulcus and tongue. These findings correlated strongly with the habitual site of tobacco placement within the oral cavity (**Table 2**). The table demonstrates that prolonged and frequent exposure to smokeless tobacco significantly increased the risk of OSCC. Individuals consuming tobacco more than 10 times daily and for over 10 years showed markedly higher disease prevalence. Histopathological grading revealed that moderately differentiated squamous cell carcinoma was the most common subtype. Poorly differentiated carcinoma was significantly associated with prolonged duration and heavy tobacco consumption. The results indicate a direct correlation between increasing duration and frequency of smokeless tobacco use with higher histopathological grade. Heavy tobacco users exhibited greater incidence of poorly differentiated carcinoma, suggesting aggressive tumor biology associated with chronic carcinogen exposure (**Table 3**). Overall, the study established a statistically significant relationship between smokeless tobacco consumption and development of OSCC. Chronic exposure, higher daily frequency and combined tobacco habits were strongly associated with disease severity and poor differentiation.

Table 1: Demographic profile and tobacco consumption pattern

Parameter	OSCC Cases (n=100)	Controls (n=100)	p-value
Mean age (years)	52.6 ± 11.4	47.2 ± 10.8	0.003*
Male gender	76 (76%)	71 (71%)	0.421
Smokeless tobacco users	84 (84%)	38 (38%)	$<0.001^*$

Gutkha users	42 (42%)	14 (14%)	<0.001*
Khaini users	25 (25%)	12 (12%)	0.018*
Betel quid with tobacco	17 (17%)	9 (9%)	0.091
Combined smoking habit	39 (39%)	18 (18%)	0.001*

Table 2: Association of duration and frequency of smokeless tobacco use with OSCC

Variable	OSCC Cases (n=84 users)	Controls (n=38 users)	p-value
Duration <5 years	9 (10.7%)	14 (36.8%)	<0.001*
Duration 5–10 years	27 (32.1%)	17 (44.7%)	0.041*
Duration >10 years	48 (57.1%)	7 (18.4%)	<0.001*
Frequency <5 times/day	12 (14.3%)	18 (47.4%)	<0.001*
Frequency 5–10 times/day	33 (39.3%)	13 (34.2%)	0.566
Frequency >10 times/day	39 (46.4%)	7 (18.4%)	0.002*
Buccal mucosa involvement	49 (49%)	—	—
Gingivobuccal sulcus	28 (28%)	—	—
Tongue involvement	15 (15%)	—	—

Table 3: Histopathological grade and tobacco exposure

Histopathological Grade	Cases (%)	Mean Duration of Habit (years)	Heavy Users (%)	p-value
Well differentiated SCC	36 (36%)	8.2 ± 2.4	13 (36.1%)	0.011*
Moderately differentiated SCC	47 (47%)	11.6 ± 3.1	28 (59.6%)	0.003*
Poorly differentiated SCC	17 (17%)	15.2 ± 4.7	14 (82.3%)	<0.001*

Discussion:

Oral squamous cell carcinoma (OSCC) remains a significant public health problem around the world, especially in India where there is a high prevalence of smokeless tobacco use. The present study showed a strong and statistically significant association between the use of smokeless tobacco and the development of OSCC, thus confirming the role of smokeless tobacco as an etiological factor in oral carcinogenesis. Rumgay *et al.*, in their study estimated that a significant number of oral cancers worldwide are caused by smokeless tobacco and areca nut use, particularly in South Asian countries where chewing is common practice, [1] and Kulothungan *et al.* in their systematic review and meta-analysis showed that tobacco use is one of the most important modifiable risk factors for oral malignancy in India [2]. The present study found that 84% of OSCC patients used smokeless tobacco regularly, with significantly more patients using smokeless tobacco than controls. The high prevalence of use of gutkha, khaini, zarda and betel quid with tobacco in India could be due to social acceptance, easy availability, low price, peer influence and nicotine dependence. Social acceptability, availability, low cost, peer influence and nicotine dependence could be the reasons for the high prevalence of use of gutkha, khaini, zarda and betel quid with tobacco in India. These factors play a significant role in the increasing incidence of oral cancer in both rural and urban population. In the current study, among the different types of smokeless tobacco products tested, gutkha was found to be the most prevalent tobacco product among the OSCC patients. These carcinogens cause oxidative stress, epithelial damage, chromosomal instability and DNA mutations that eventually lead to malignant transformation of oral epithelial cells, as reported by Khan *et al.* [5] and Nikam *et al.* [6] who have seen that commercially available smokeless tobacco products contain highly variable amounts of tobacco specific nitrosamines, heavy metals and areca nut alkaloids, which increase the risk of oral cavity cancer in chronic smokeless tobacco users. In the present study, there was a significant association between duration and frequency of smokeless tobacco use and OSCC. The prevalence

of carcinoma was significantly higher in patients who chewed tobacco for more than 10 years and had more than 10 chewings of tobacco per day. The results are similar to those of the systematic review conducted by Asthana *et al.*, which showed a dose-response relationship between the frequency of smokeless tobacco use and oral cancer risk [3]. Chronic exposure of the mucous membranes to carcinogenic compounds results in progressive epithelial dysplasia, build-up of genetic mutations and formation of invasive carcinoma. Chami *et al.* also noted that chronic inflammation, generation of reactive oxygen species and irreversible damage of cells are promoted by the prolonged exposure to the components of smokeless tobacco [9]. In the present study, buccal mucosa was found to be the most frequent site of OSCC followed by gingivobuccal sulcus and tongue. This distribution is similar to the normal location of the tobacco quid in the oral cavity. Repeated local irritation and chronic exposure of the mucosa to tobacco carcinogens leads to hyperkeratosis, epithelial atrophy, dysplasia and invasive squamous cell carcinoma. Warnakulasuriya also noted that oral potentially malignant disorders at quid placement sites have high malignant transformation potential [10, 14] and Indian studies reported similar anatomical distribution patterns with high prevalence of gingivobuccal complex cancers in smokeless tobacco users [7]. The present study also confirmed that heavy smokeless tobacco exposure was significantly related to poorly differentiated squamous cell carcinoma. There was a greater prevalence of advanced histopathological grades in patients with longer duration and more frequent chewing habits. Li *et al.* reported that tobacco exposure promotes oral carcinogenesis by p53 mutation, EGFR dysregulation, activation of inflammatory cytokines, disruption of the cyclin pathway and genomic instability, which all play a role in both the initiation and aggressive progression of oral tumors, metastasis and poor prognosis [11]. Caruntu and Caruntu also reported that tobacco-induced molecular alterations are not only involved in the initiation of tumors but also in the aggressive progression of tumors, metastasis and poor prognosis [13]. WHO estimates indicate that India remains one of the largest consumers of

smokeless tobacco globally, with millions of adults regularly consuming chewing tobacco products [10]. Recent epidemiological analyses by Goyal *et al.* showed a rising prevalence and mortality trend of head and neck cancers in India, particularly among populations with high tobacco exposure [12]. Rumgay *et al.* further estimated that nearly one-third of oral cancer cases worldwide are directly attributable to smokeless tobacco and areca nut consumption [1]. These findings collectively emphasize the enormous preventable burden associated with chewing tobacco habits. The other significant finding of the current study was that combined smoking and chewing was more frequent among OSCC patients. Combined use of smoked tobacco, smokeless tobacco and alcohol is a significant oral cancer risk because of the cumulative exposure to carcinogens. Tobacco-related head and neck cancers have been described earlier by Gupta *et al.* as an ongoing problem in the world, mainly due to the combined effect of lifestyle-related risk factors [15] and also in a few Indian and international studies that have studied oral carcinogenesis among chronic tobacco users [2, 7]. There are some limitations in the present study. The results obtained may not be representative of the general population as it was performed in a single tertiary care centre. Data may also be inaccurate due to recall bias for duration and frequency of tobacco use. Besides, other contributing factors, like diet, viral infections and genetic susceptibility, were not studied in detail. However, the study has ample evidence to support the strong etiological association of smokeless tobacco with the development of OSCC in the Indian population. Public health interventions to curb the use of smokeless tobacco are needed now. Oral cancer incidence can be significantly decreased through awareness campaigns, school-based educational programs and early detection of potentially malignant disorders, tobacco cessation counseling and enforcement of tobacco control laws. Behavioral modification and early diagnosis continue to be key to reducing the morbidity and mortality of smokeless tobacco-related oral malignancies.

Conclusion:

Smokeless tobacco use was significantly associated with the occurrence of oral squamous cell carcinoma, with gutkha and khaini being the most commonly used products. Longer duration and higher frequency of use were associated with greater disease severity and advanced histopathological grade, with buccal mucosa being the most common quid placement site. Early identification of high-risk individuals strengthened tobacco-control policies and targeted awareness programs are essential to reduce smokeless tobacco-associated oral cancer.

References:

- [1] Rumgay H *et al.* *Lancet Oncol.* 2024 **25**:1413. [PMID: 39393386]
- [2] Kulothungan V *et al.* *JCO Glob Oncol.* 2024 **10**:e2400152. [PMID: 39173081]
- [3] Asthana S *et al.* *Tob Prev Cessat.* 2019 **5**:34. [PMID: 32411897]
- [4] Parihar AS *et al.* *J Pharm Bioallied Sci.* 2024 **16**:S3768. [PMID: 39926943]
- [5] Khan SZ *et al.* *Turk J Med Sci.* 2020 **50**:291. [PMID: 31655504]
- [6] Nikam S *et al.* *Lancet Reg Health Southeast Asia.* 2024 **29**:100457. [PMID: 39258248]
- [7] Sultana A *et al.* *J Indian Assoc Public Health Dent.* 2024 **22**:355. [DOI: 10.4103/jiaphd.jiaphd_41_24]
- [8] Pullat GB *et al.* *Int J Cancer.* 2026 **159**:368. [PMID: 41657059]
- [9] Chami A *et al.* *Sci World J.* 2026:3369964. [PMID: 42032931]
- [10] Warnakulasuriya S *et al.* *Oral Dis.* 2021 **27**:1862. [PMID: 33128420]
- [11] Li J *et al.* *Cureus.* 2024 **16**:e66966. [PMID: 39280415]
- [12] Goyal AK *et al.* *Indian J Surg Oncol.* 2025 **16**:1590. [PMID: 41415757]
- [13] Caruntu A & Caruntu C. *J Clin Med.* 2022 **11**:6406. [PMID: 36362637]
- [14] Warnakulasuriya S. *Oral Surg Oral Med Oral Pathol Oral Radiol.* 2018 **125**:582. [PMID: 29673799]
- [15] Gupta B *et al.* *Oncology.* 2016 **91**:13. [PMID: 27245686]

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